CHARACTERIZATION OF ATHEROSCLEROTIC PLAQUES BY CROSS-POLARIZATION OPTICAL COHERENCE TOMOGRAPHY

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ABSTRACT

We combined cross-polarization optical coherence tomography (CP OCT) and non-linear microscopy based on second harmonic generation (SHG) and two-photon-excited fluorescence (2PEF) to assess collagen and elastin fibers in the development of the atherosclerotic plaque (AP). The study shows potential of CP OCT for the assessment of collagen and elastin fibers condition in atherosclerotic arteries. Specifically, the additional information afforded by CP OCT, related to birefringence and cross-scattering properties of arterial tissues, may improve the robustness and accuracy of assessment about the microstructure and composition of the plaque for different stages of atherosclerosis.

Keywords: Cross-Polarization Optical Coherence Tomography (CP OCT), atherosclerotic plaque (AP), non-linear microscopy, collagen and elastin fibers

1. INTRODUCTION

Development of high resolution imaging technologies opened new possibilities for studying spatial organization of tissues in different pathologies. Inflammation, typical for atherosclerosis, disorganises collagen and elastin fibers, which, in turn, affects polarization light propagating through the tissue and can be used to characterize the atherosclerotic plaque (AP). The existence of commercially available intravascular optical coherence tomography (IV OCT) systems, as well as high quality research systems for IV OCT, has stimulated a large number of pre- and clinical research efforts, however the identification of different development stages for AP remains a challenge. In particular, the Consensus Standards paper [1] recommends further *ex vivo* studies with histological confirmation to establish and validate reliable criteria for plaque characterization.

IV "non-polarization sensitive" OCT (although OCT is intrinsically sensitive to the polarization changes during propagation in tissue or/and backscattering) was studied by many researchers to evaluate the thin-capped fibroatheromas (TCFAs) [2-6]. However, the accumulated experience demonstrated that the contrast between the fibrous cap (FC) and necrotic core (NC) of the plaque is often insufficient to visualize these structures. It was hypothesized that polarization sensitive OCT (PS OCT) (where the information about polarization changes is intentionally acquired and extracted, typically by registration of two polarization channels) should be able to image these structures using birefringence properties, visualized as phase retardation images [5, 7-11], since phase retardation change with depth should characterize orientation and level of structural organization of the collagen at the fiber and tissue level [10]. It was also expected that this approach could be successfully used to visualize the large vessel walls [12-14]. However this

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methodology has limitations. One is that the small capsule thickness in the case of the vulnerable AP (less than ~65 μm) is not sufficient to accumulate measurable phase retardation (delay between eigen polarizations). Another drawback is that although phase retardation is relatively straightforward to visualize and measure in homogenous birefringent media, this is not so in vessels and plaques. That is, real biotissue has various structures of a similar scale, and intrinsic backscattering contrast between these structures creates additional brightness modulation which is difficult to distinguish from the phase retardation. We will illustrate this potential ambiguity in greater detail below. Cross-polarization OCT (CP OCT) is variant of PS OCT with some advantages over "conventional" implementations, hence we perform an *ex vivo* coronary artery study using it. The results will form a useful dataset and springboard [23] for potential *in vivo* work. Along the way, we resolve the birefringence / cross-polarization ambiguity. Specifically, we combine CP OCT and non-linear microscopy based on second harmonic generation (SHG) and two-photon excitation fluorescence (2PEF) to assess collagen and elastin fibers and other vascular microstructures in the development of the AP. We are proposing the CP variant of PS OCT to better address the outstanding issues in vulnerable plaque assessment.

2. MATERIALS AND METHODS

The objects under study were *post mortem* samples of coronary arteries of patients (in the age range 65-90) died from cardiovascular disease. The arterial vessels obtained not later than 24 hours after death were cut into fragments with length of 1-2 cm and delivered to the laboratory in gauze moistened with phosphate buffer at 7°C.

The spectral domain CP OCT device designed and developed at the Institute of Applied Physics of the Russian Academy of Sciences (Nizhny Novgorod) [15-18] was employed in the study. The light source was a superluminescent diode with a central wavelength of 1310 nm, radiation power of 20 mW and a spectral width of 100 nm, resulting in axial resolution of 10 μm. The system had active, closed loop control of the polarization at the fiber output, enabling maintenance of circular polarization state emerging from the fiberoptic probe; this is true even in the presence of probe bends and resultant changes in the fiber birefringence. The format of OCT images presentation in all figures in this paper is as follows: top image shows co-polarization OCT channel, the middle panel is the cross-polarized data, and the bottom display is a Pythagorean sum of the two polarization channels above (representing "true polarization insensitive" image).

The CP OCT images were obtained from the intima side of fresh arterial samples (within 2 hours after excision). The lateral scanning in the images was directed along the vessel and the lateral scanning range was either 2.7 or 4 mm, depending on the plaque size. After the OCT imaging, we marked the OCT probe area (6 mm in diameter) on the specimen with an ink circle and the specimen were fixed (10 % formalin for 48 hours with standard paraffin embedding process) and serially sliced in the central area, with the slice location matching the OCT imaging plane. Serial sections 10 μm thick we used for non-linear microscopy and 7 μm thick were used for histological studies (serial sections were stained with H&E, Van Gieson and Orcein).

Visualization of the collagen and primary elastin fibers in the walls of the vessels was conducted on a confocal laser-scanning microscope (LSM 710, Carl Zeiss, Germany). Detection was performed simultaneously in two channels by using filters in the wavelength ranges 362-415 nm and 480-554 nm, with the aim of obtaining signals indicating SHG emission (green color in the images) and 2PEF emission (red color in the images), respectively.

Thus the image set for each specimen included 3 modalities obtained from the same area – CP OCT, non-linear microscopy and histopathology. 63 different regions of interest were studied in total. All cases were evaluated and confirmed by according to the American Heart Association (AHA) criteria [19, 20] and "Virmani's vulnerability criteria" [21, 22].

3. RESULTS

3.1. Intimal thickening of coronary artery, with lipid pool

As atherosclerosis progresses, we can see lipid patches or streaks (lipid pools) within the thickening intima. According to the American Heart Association (AHA) classification, this represents Stage II and/or intermediate Stage III

in the development of the atherosclerosis [19, 20]. Generally, the lipid pool is formed by the accumulation of lipid-laden foam cells (Fig.1A-C, H). Slight disruption of the collagen and elastin fibers of the intima can be observed in the form of weak fibrinoid swelling of the collagen fibers and partial fragmentation of the elastin fibers (Fig. 1A and B, respectively).

As seen in the non-linear microscopy images, there is a brighter SHG signal from the collagen (Fig. 1D) and a less bright 2PEF signal from the elastin (Fig. 1E) in the intima. A lipid pool located in the deeper regions of the thickening intima can be seen as lacking in 2PEF-SHG signals (Fig.1F, arrow), consistent with the accumulation of the foam cells evident on the histological slide (Fig. 1C, arrow). Because of the paraffin embedding process, the solvent treatment removes the lipid from these lipid-loaded structures, which therefore appear as empty spaces in sections.

The CP OCT image shows the entire thickened vessel wall (about 600 μm), and in the co-polarization the layered structure of the outer layers of the vessel wall is almost completely lost (Fig. 1G, top image). It is evident that the thickened intima has a high-level and homogeneous OCT signal (Fig. 1G, green arrow), not contrasting with the area of the lipid streak on the histology (Fig.1 A-C). This means that this stage is practically indistinguishable on CP OCT from the stage without lipid streaks, which is important for clinical applications. the OCT image in the cross-polarization channel (Fig. 1G, middle image, arrow) has a polarization artifact (fringe) which can be interpreted as visualization of the lipid streak (histologically located on the same depth to Fig. 1C, arrow). However it is most likely a polarization fringe related to the phase retardation between two eigen polarizations, as can be seen on the sum image where this "structure" completely disappears (Fig.1G, bottom image, square).The absence of this "feature" in the Pythagorean sum image is a good indication that it is a birefringence-related artifact.

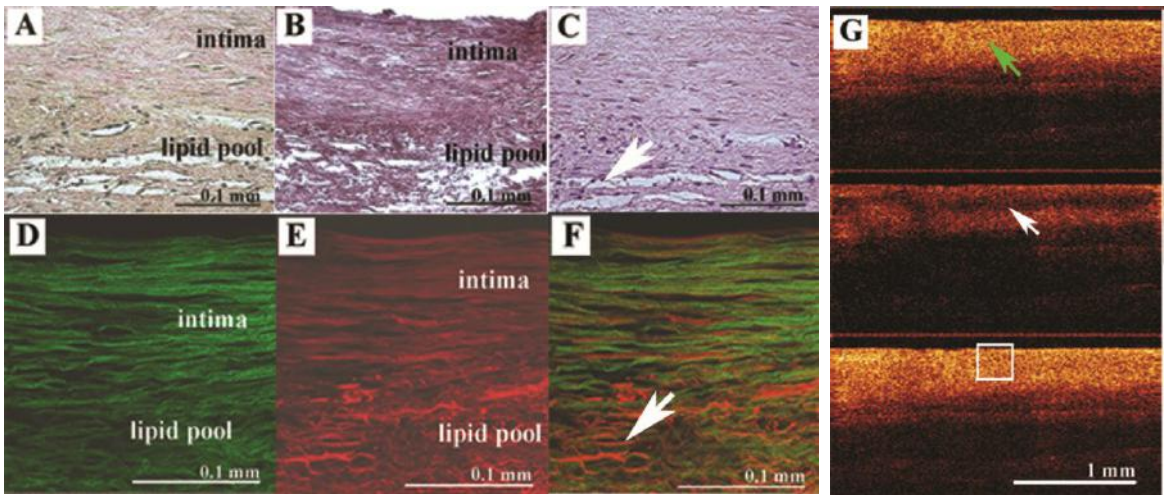

Fig. 1. The stage of lipid pool in the intimal thickening coronary artery. Histological slides stained with Van Gieson (A), Orcein (B) and H&E (C). SHG (D), 2PEF (E) and combined SHG-2PEF (F) images. CP OCT image (G). Foam cells (arrow) in the intima in (C) which form the lipid streak with a weak OCT signal in cross-polarization (white arrow) and a high-level homogeneous OCT signal in co-polarization (green arrow) in (G). Here and after: top = co-polarization CP OCT image, middle = cross-polarization CP OCT image, bottom = Pythagorean sum of the two polarization channels — the bottom image. The images in (A)–(F) correspond to the region indicated by squares in (G) [23].

3.2. "Mature" vulnerable atherosclerotic plaque

This is classified as Va stage according to the AHA [19, 20]. It is known that AP can get to this stage only in the presence of active inflammation, when the collagen and elastin fibers of the FC undergo considerable disruption due to the action of proteolytic enzymes. This is the reason for the formation a vulnerable AP with a thinned FC (thickness of <65 μm), infiltrated foam cells and inflammatory cells with a large mature NC occupying more than 40% of the whole area of the AP (according to the "Virmani's criteria" [21, 22]).

On histology the accumulations of foam and inflammatory cells at the border of the FC and the NC are clearly visible (Fig. 2C, arrow). This is easily detectable in non-linear microscopy images as regions lacking a signal, corresponding to

the accumulation of these cells, therefore accurately reflecting the shape of this tissue compartment (Figures 2D-F, arrows). The SHG and 2PEF signals demonstrate the presence of highly disrupted collagen and elastin fibers in the area of the thin FC (Fig. 2D and E, respectively).

In the CP OCT image in the co-polarization the upper layer of the FC over the NC is visualized as an area with a highly heterogeneous OCT signal, quickly decreasing with depth (Fig.2G, top image). In the cross-polarization, the corresponding area shows weak heterogeneous OCT signal depolarization (Fig. 2G, middle image). This indicates the prevalence of disrupted fibers in the FC and during analysis of the image it can serve as an indicator of AP instability. Such components of AP as the accumulation of cells located between the lower part of the FC and the surface of the NC are capable of effective depolarization of the radiation. These components are visualized in the cross-polarization CP OCT channel as “bright spots” (Fig. 2G, middle image, arrow). The accumulations of cells can considerably weaken the probing and backscattered radiation, generating a shadow – the occurrence of the area with a weak signal making it difficult to visualize the NC. The accumulations of cells are hard to differentiate from microcalcification deposits on the surface of the AP.

The area of the AP with a weak or completely absent signal, and with poorly defined borders and rapid weakening of the OCT signal with depth in the CP OCT image in both the co- and cross-polarizations (Fig. 2G, top and middle images) corresponds to the area of the large, mature NC (Fig. 2H). The larger size of the NC (thickness up to 500 μm), its content in the form of lipid-protein detritus and the poor imaging depth do not allow its complete visualization in the CP OCT image.

The cases studied to date suggest that the visual analysis of OCT signals in the cross-polarization image can differentiate the state of the unstable FC in the AP of coronary arteries (Fig. 2G, middle image).

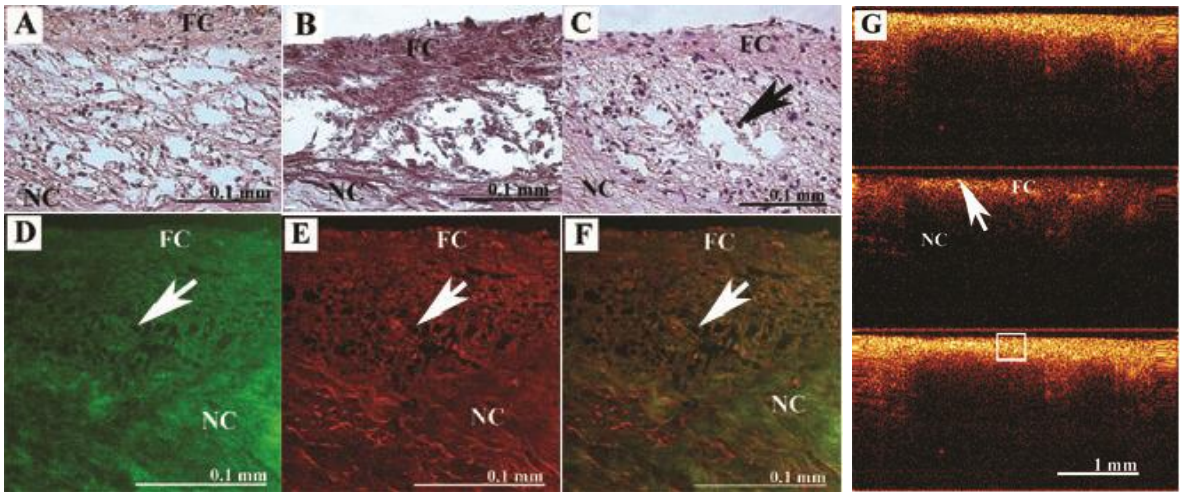

Fig. 2. The stage of “mature” vulnerable atherosclerotic plaque. Histological slides stained with Van Gieson (A), Orcein (B) and H&E (C). SHG (D), 2PEF (E) and combined SHG-2PEF (F) images. CP OCT image (G). FC - fibrous cap, NC- necrotic core. The accumulations of foam and inflammatory cells (arrows) at the border of the FC and the NC in (C). The images in (A)–(F) correspond to the region indicated by squares in (G) [23].

3.3. “Mature” stable atherosclerotic plaque

This stage is characterized by a moderately thickened and tear-resistant fibrous-type AP (Vc stage according to the AHA classification). On histology, moderately thickened and well-ordered collagen and elastin fibers are clearly visible in the area of the highly thickened FC of the AP (Fig. 3A and B, respectively). That is the reason for the frequently observed clinical narrowing of the vessel lumen. In this AP, the NC is typically of small size, and in most cases is not clearly visible on histology. Non-linear microscopy provided both a high level of SHG signal from the collagen and of the 2PEF signal from the elastin (Fig. 3D and E, respectively), demonstrating their thickness (up to 8 μm), high density of packing, and the ordered arrangement in the area of the thickened FC (Fig. 3F).

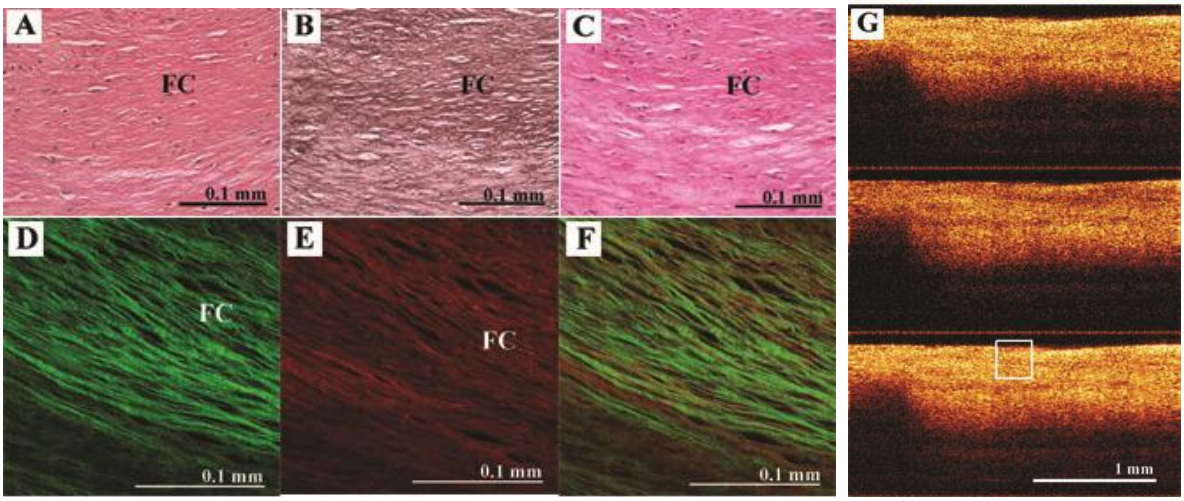

Fig. 3. The stage of "mature" stable atherosclerotic plaque. Histological slides stained with Van Gieson (A), Orcein (B) and H&E (C). SHG (D), 2PEF (E) and combined SHG-2PEF (F) images. CP OCT image (G). FC - fibrous cap. The images in (A)–(F) correspond to the region indicated by squares in (G) [23].

CP OCT imaging shows a pattern of alternating bright and dark stripes in both polarizations to a considerable depth (about 1 mm) (Fig. 3G). This pattern could resemble birefringence fringes, but since it is preserved in the sum channel, it is likely a real backscattering pattern.

4. DISCUSSION AND CONCLUSION

The present study demonstrates that CP OCT can identify intimal disruption, as well as large lipid deposits, suggesting that CP OCT may detect important microstructural features associated with vulnerable plaques. It enables visualization of the plaques structural characteristics, and combined with non-linear microscopy and histology, aids in understanding important tissue properties such as integrity, degradation, orientation, packing density of collagen and elastin fibers, as well as degree of inflammation of the fibrous capsule. CP OCT signal clearly reflects the degree of change in major components of AP polarization properties, which in turn may be indicative of the functional status of the AP. For example, the high cross-scattering (Fig. 3G), similar to findings of the early works by PS OCT [3,5,7] showed a high content of thick and organized collagen fibers of fibrous plaques, suggesting an increase of stability and thickness FC plaques. It is shown that in the disorganized state, collagen and elastin fibers are unable to effectively change the light polarization (Fig. 2G). At the same time, the typical heterogeneous cross-scattering in the form of "bright spots", with the prevalence of the disorganized fibrous structures in inflammation, indicate an increase in the vulnerability of the plaque (Fig. 2G). We cannot tell exactly the corresponding histological nature of these "bright spots" - it could be regular macrophages, giant cells or cholesterol crystals, as demonstrated in [24] or other cell clusters (such as T-lymphocytes and foam cells), but in any case we consistently see this "bright spots" pattern as characteristic for this stage of the AP.

Specifically, the additional information afforded by CP OCT, related to birefringence and cross-scattering properties of arterial tissues, may improve the robustness and accuracy of assessment about the microstructure and composition of the plaque for different stages of atherosclerosis. However it can also create a difficulty with the image interpretation, because backscattering contrast may have a similar appearance to the birefringence related fringes. They can be separated using Pythagorean sum of the channel, there only backscattering-related contrast sustain.

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